

Plasmid miniprep (Mark)

1. Spin down your 5 ml culture, 1 min 6K. Pour off supernatant.
2. Resuspend in 200ul **Solution I** with 100 ug/ml RNase A (1:100 dilution of 10 mg/ml RNase A stock)
3. Add 200ul **Solution II**; invert gently 5-6 times until well mixed. Do not vortex! Allow to go clear but no longer than 3-5 minutes.
4. Add 200ul **Solution III**. Invert gently until cloudy.
5. Spin 5 mins max speed to pellet debris.
6. Remove pellet with sterile toothpick.
7. Add 500 ul chloroform (or 24:1 chloroform:IAA). Vortex well until cloudy.
8. Spin 2 mins max speed.
9. Remove top layer to a fresh microfuge tube.
10. Add 500 ul (or equal volume) isopropanol. Mix well and let sit 2-5 mins room temp.
11. Spin 15 mins max speed.
12. Remove supernatant and wash well with 500 ul 70% EtOH.
13. Air dry 5 mins; resuspend in 30ul **Elution Buffer**.

Solution I

10mM Tris-HCL pH 8, 1 mM EDTA

Solution II

0.2M NaOH, 1% SDS. Make fresh!

Solution III

3M KOAC pH 4.8. Dissolve in very little water, add acetic acid to pH

Elution Buffer

10mM Tris pH 8.5